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DEVELOPMENTAL TOXICITY (DOMINANT LETHAL MUTATION) STUDY ON AGENT LEWISITE

Dominant Lethal Study of Lewisite in Male Rats

FINAL REPORT

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December 1993

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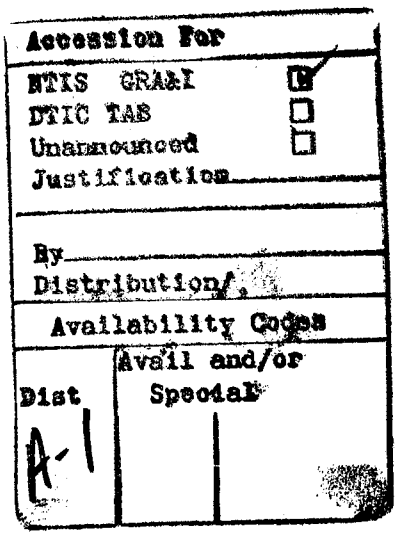

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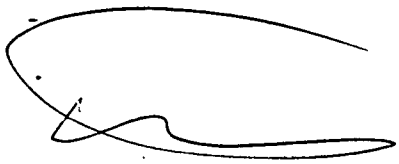
Dominant Lethal Study of Lewisite in Male Rats

FINAL REPORT



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EXECUTIVE SUMMARY

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INTRODUCTION

Chemical warfare agents present long-term environmental or occupational health hazards for workers in operations involving these compounds. Lewisite (dichloro(2-chlorovinyl) arsine) presents a potential for accidental or occupational exposure because it is used in a number of research laboratories, stored in depot sites throughout the country and occasionally is transported to distant sites. In addition, stockpiles of Lewisite are scheduled for destruction by the US Army in the near future, creating an additional potential for environmental and occupational exposure. Although considerable information is known concerning the acute effects of Lewisite[11,14], few data are available on its long-term hazards. This concern has prompted this study, to identify the potentially toxic, mutagenic and reproductive effects of Lewisite and to establish a database for the development of hazard evaluations and occupational health standards for this chemical.

Lewisite is a highly toxic chemical vesicant that reacts with the sulfhydryl groups of proteins through its arsenic group.[6,14] In the presence of water or alkalies, Lewisite hydrolyses to form Lewisite oxide, which is non-volatile and insoluble in water. Although few data are available, Lewisite oxide is generally thought to be a weaker vesicant than the parent compound. Relevant chemical and physical data for Lewisite are summarized in the Materials and Methods section.

Comprehensive reviews are available on chemical and toxicity data about Lewisite in animals and humans, including that acquired during World War I and World War II.[11,14] Exposure to Lewisite is characterized by the immediate onset of pain, with the mucus membranes of the respiratory and gastrointestinal tracts particularly susceptible to Lewisite. Lewisite is both a lethal vesicant and a systemic toxin; the liver, kidneys, gall bladder, bile ducts and other organs are vulnerable to injury if absorption occurs.[5] Inhalation of Lewisite vapor produces pulmonary edema and accumulation of pleural fluid.[11,14] Skin contact with liquid Lewisite produces vesication, edema and necrosis. Fatal systemic intoxication was evident in dogs a few hours after application of Lewisite to the skin. Although the immediate cause of death was not apparent, fluid losses due to change in capillary permeability caused large decreases in blood volume[11].

In a 90-day subchronic study of Lewisite administered by gavage to Sprague-Dawley CD rats, a combined mortality of 30% was reported for doses of 0.5, 1.0 and 2.0 mg/kg. Serum protein, creatinine, SGOT and SGPT were decreased in surviving males but not females. Many of the survivors also had ulcers of the forestomach and inflammation of the glandular stomach. The forestomach ulceration was considered to be the major effect attributable to exposure. Respiratory tract inflammation occurred in most exposed animals and was the cause of death in most non-survivors. The no-effect dose was between 0.5 and 1.0 mg/kg. [29]

Few data are available to evaluate the potential chronic effects of Lewisite other than anecdotal evidence from war use. Based on one incident of accidental exposure, Lewisite is suspected to be carcinogenic in man[19]. Japanese factory workers producing mustard and Lewisite agents during World War II had a high mortality rate due to

respiratory and gastrointestinal cancers[26,32,34]. However, because these workers were potentially exposed to unknown quantities of both sulfur mustard and Lewisite, among other compounds, it is not possible to implicate Lewisite specifically as a carcinogen since sulfur mustard is a known carcinogen.

Data on the mutagenicity of Lewisite are limited. No mutagenic response was found in the fruit fly[3], root tip[21] or Ames and Chinese hamster ovary cell assays[18,31]. A Segment II teratology study of Lewisite suggested that Lewisite is not teratogenic in the rat or the rabbit after short term exposures, since fetal effects were observed only at dose levels that induced maternal toxicity[17].

Many of the symptoms of intoxication by Lewisite and arsenic are similar, including severe inflammation of the gastrointestinal tract associated with electrolyte disturbances and ulceration and perforation of membranes, suggesting that the systemic toxicity of Lewisite may result from its arsenic group[22]. Arsenic, as sodium arsenate or arsenite, is known to be embryotoxic and teratogenic in a number of animal species[21,24]. A comparison of Lewisite and sodium arsenite toxicity in the rabbit following intravenous administration revealed that the LD_{50} values for sodium arsenite and Lewisite were different, 7.6 and 1.8 mg/kg, respectively[12]. Furthermore, significant differences in tissue arsenic content and pathology were reported for the two chemicals. It was estimated that the arsenic uptake and accumulation from Lewisite exposure in maternal animals and their fetuses would not be significant at non-lethal doses in short-term teratology studies[17]. However, arsenic accumulation may be important in long-term exposures. Comprehensive data are not available to evaluate the potential risk to reproduction from long-term occupational exposure to Lewisite.

The male dominant-lethal test evaluates the potential for genetic toxicity of a substance after administration of the substance to the male. Adult male rats were dosed acutely with sublethal concentrations of Lewisite. These treated males were then mated with different pairs of untreated females each week for 10 consecutive weeks. The results of the matings can then indicate the specific stages of male gametogenesis that are affected and responsible for any resultant embryonic mortality. Reproductive deficiency in matings during Weeks 1 and 2 represent effects on mature spermatozoa; Weeks 3, 4, and 5 on spermatids; Weeks 6, 7, and 8 on spermatocytes; Weeks 9 and 10, on differentiating spermatogonia. There are multiple reviews of the theory and design of the dominant-lethal test[1,4,8,9,15,16,20], and at least one of the sperm morphology test[33]. The genetic effects of ethyl methanesulfonate, the positive control in the current studies, have also been reviewed[30].

MATERIALS AND METHODS

Relevant Chemical and Physical Data for Lewisite, dichloro(2-chlorovinyl)arsine[28]

CAS number: 541-25-3
RTECS number: CH2975000
Structure: Cl-CH=CH-AsCl2 dichloro(2-chlorovinyl)arsine
Molecular weight: 207.3 g
Density at 20°C: 1.888 g/ml
State: Dark, oily liquid (stable in steel and glass)
Vapor pressure at 20°C: 0.394 mm; Hg at 25°C
Decomposition temperature: >100°C
Solubility in water: Very slightly soluble
Hydrolysis Products
chlorovinyl arsenous oxide, HCl (in acid solutions)
acetylene, sodium arsenate (in alkaline solutions)

Maximum Tolerated Dose

The amount of test compound to be used as maximum tolerated dose (MTD), 1.5 milligrams per kilogram body weight, was provided by the sponsor.

Characterization of the Test Article(s)

The test compound was analyzed by LTC Theodore Dolyine, Chief, Analytical Chemistry Branch, US Army Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010 (4 Dec 89). Based on nuclear magnetic resonance and gas chromatography, the material was specified to be 96.1% pure, and was supplied as 30 ml of a concentration of 1.5 mg/ml in sesame seed oil. The material was received on 20 Dec 89 and stored at -70°C until used.

Positive Control Materials

Ethyl methanesulfonate [EMS], (Sigma Chemical Co., St. Louis, MO) CAS# 62-50-0, a known mutagen was used as positive control treatment. EMS was determined to be >99% pure ethyl ester of methanesulfonic acid, by the Division of Analytical Chemistry, NCTR. Analysis was by flame ionization gas chromatography, confirmed by mass spectrometry . The <1% impurity was identified as hexachloroethane and was interpreted to be a contaminant from the chloroform solvent used in the analysis. Periodic reanalyses were performed to verify concentration and stability.

Selection and Characterization of Diluent (Vehicle)

Sesame seed oil was selected as the vehicle, in accord with the precedent established in earlier studies of Lewisite [29]. These investigators quoted Hackett[17] in substituting sesame seed oil for the usual corn oil, to avoid phytosteroids in the corn oil. Sesame seed oil is "generally recognized as safe" by the FDA [10], it is readily available, contains no preservatives and appears quite stable. The sesame seed oil was produced

by the Hain Pure Food Company, Los Angeles, CA 90061 (Bar Code Label #23254114211) and was purchased locally.

Route of Exposure

The oral route of exposure was specified by the sponsor for this study. The expected routes of human environmental exposure to Lewisite are inhalation, dermal exposure, or by ingestion either directly or from swallowing exhaled material. It was considered impractical to expose the rats by inhalation because of the potential hazards to personnel, technical aspects of generating the aerosol and the cost of a long-term inhalation exposure. Direct application to the skin was not selected because of hazards incurred while handling the animals and the possible development of lesions which could cause systemic translocation of material. Injection of the material was ruled out because of the potential of local lesions from multiple injections of the agent. All animals were assigned randomly by weight rank (from heaviest to lightest) within sex to treatment groups and cages. The doses and dose groups were identified by color code, with technicians unaware of the amount of compound represented by each color.

EXPERIMENTAL DESIGN

This Dominant Lethal Protocol met or exceeded the proposed United States Environmental Protection Agency Guidelines for Assessing Male Reproductive Risk (1988) [Federal Register, 53(126):24850 - 24869] and the proposed United States Environmental Protection Agency Guidelines for Assessing Female Reproductive Risk (1988) [Federal Register, 53(126): 24834 - 24847] regarding requirements for number of animals per treatment group, number of treatment groups, selection of dose levels, dosing period, study duration, reproductive end points evaluated, record management, histopathological evaluation and statistical analysis. The study was conducted in compliance with the EPA and FDA Good Laboratory Practice Regulations. This protocol also met the United States Environmental Protection Agency Guidelines for the Health Assessment of Suspect Developmental Toxicants:

> Final Rules (1986) [Federal Register, 51(185): 34028 - 34040] and the United States Environmental Protection Agency Toxic Substances Control Act Test Guidelines: Final Rules (1985) [Federal Register, 50(188): 39426 - 394361].

The animals used were Sprague-Dawley CD rats from the NCTR breeding colony (NCTR:523 (SD)), 11-12 weeks of age. The females were housed two per cage, the males singly, in polycarbonate cages that were 17"x8¼"x8". Hardwood chips (Northeastern Products Corp, Warrensburg, NY) were used for bedding; cages were changed weekly. The animal room was maintained at 23.0±3.0°C and 50±10% relative humidity. The light was controlled to provide twelve continuous hours each of light and dark. The animals had unlimited access to filtered water and an open formula ration (NIH-31, #5022, Purina Mills, St. Louis, MO.). The animals were identified by clipped ears and cage cards.

The Sprague Dawley genotype was selected because of its established fecundity and its extensive historical database of preceding reproductive studies at NCTR.

Twenty males per dose level were treated by oral gavage with either 0.00, 0.375, 0.750 or 1.500 mg/kg of Lewisite (1.5mg/ml in sesame seed oil) for five consecutive days. Twenty positive control males were given one ml sesame seed oil by gavage for four days; on the fifth day they were given ethyl methanesulfonate intraperitoneally (100 mg/kg). The EMS dose preparation contained 60 mg/ml in sesame seed oil. Twenty additional males were gavaged for five days with one ml sesame seed oil only, and served as negative controls. Dosing was conducted during the period 3-12 January, 1990. A locally developed automated dosing instrument was used to gavage the animals. The instrument had been described previously[7] and used extensively. It consists of a computer-driven, two-barrelled automatic pipettor that obtains the body weight of the animal via an interfaced balance, then delivers the appropriate mixture of agent and diluent through a gavage tube attached by a "Y" connector to the pipettor syringes.

Two days after treatment ended, each male was placed in a cage with two untreated virgin females for five days, and was returned to its own cage for the next two days. This schedule, with two new females each week, was repeated for ten weekly cycles. Historically, conception occurs in 85-90% of females by the end of the fifth day.

Pregnant females were euthanized with CO_2, 17 days after the start of cohabitation with the male (Days 14 to 17 of gestation). Their ovaries were examined and corpora lutea, living fetuses, dead implantations and any resorption sites were enumerated. Their brain, ovaries, uterus and pituitary gland were collected, weighed and fixed in 4% neutral buffered formalin.

The males were euthanized with CO_2 during the week following the tenth weekly cohabitation. The left testes and epididymis were removed and weighed, and epididymal sperm were removed for analyses of motility and morphology. The brain, pituitary gland, seminal vesicles and prostate were also removed, weighed and all tissues were fixed in 4% neutral buffered formalin.

The left testis from each male was fixed by immersion in 4% neutral-buffered formalin (the tunica was first punctured in several places at each pole with a 21-gauge hypodermic needle). A cross-section of the central portion of the fixed testes was embedded in glycolmethacrylate and two adjacent two-micron thick sections were cut. One was stained with the periodic acid-Schiff technique to demonstrate tubular basement membrane; this section was used to determine tubule diameter planimetrically. The second section was stained with hematoxylin and eosin for histopathologic examination.

Morphology and motility of sperm were characterized by standard techniques[13] with modification for automated analysis (CASA;Model HTM-2030, Hamilton-Thorn Research, Beverly, MA).

Preliminary results of this work have been reported (see Parker, et al. [26a,26b]).

Statistical Analyses

The reproductive criteria in the females were analyzed according to Green, et al [15,16]. The litter was used as the basis for analysis of all fetal variables. The Chi-square test compared the mean fertility index between the untreated controls and each treated group; a trend for linear proportions[2] was used to determine whether the fertility indices observed were related linearly or logarithmically to dose. A t-test was used to determine whether or not significant differences existed between the control and treated groups in mean number of implantations and regression analysis was used to determine whether any relationship to dose was linear or logarithmic. The t-test was also used to determine whether or not significant differences occurred in the average number of corpora lutea per ovary, among treated and control animals. To determine any effect on preimplantation losses or number of dead implants, a Tukey-Freeman arc-sine transformation of the data was used[23] and the resulting values were compared by t-test. The proportion of dead implants (postimplantation lethality) was determined by the formula of Rohrborn[27]. The proportion of females with one or more dead implants was compared to control values by Chi-square test, with the trend test for linear proportions used to determine if effects were linear or logarithmic with dose[2]. Probit regression analysis was also performed to determine whether or not the probit of the proportion was related to logarithmic dose. The lethal implantation index (dead implantation/total implantation) was calculated for each female. The control values were compared to each treatment value by t-test.

For the males, the sperm motility analysis estimated the actual number of motile and nonmotile sperm and the sperm count determined the sperm concentration per gram of cauda epididymis. The sperm head morphology assessment determined the percentage of abnormal sperm classified as blunt hooked, banana-shaped head, amorphous head, two heads/two tails, short sperm head, or other (i.e., multiple tails, twisted heads).

The data were transformed with the Tukey-Freeman transformation for proportions. A one-tailed t-test was used on the transformed values for each category.

Weekly animal body weights were summarized by dose level and sex according to Nelson[25].

QUALITY ASSURANCE

All aspects of the studies were conducted in accordance with Good Laboratory Practice Regulations, Food and Drug Administration (Federal Register, Vol. 52, No.172, September 4, 1987, pp. 33768 - 33782) and in accordance with written Standard Operating Procedures (SOP) of the performing unit. An administratively separate quality assurance unit (QAS) at NCTR monitored the studies to assure adherence to Good Laboratory Practices and to the approved SOPS.

STORAGE OF RECORDS

All hard copies of data sheets for the present study are stored in the NCTR Archives under the control of the NCTR Archivist officer. Biological samples collected during the course of the study are placed in secure storage in the Pathology Division, NCTR. The work sheets and computer printouts generated in the statistical analysis of data are stored at the NCTR. In accordance with Sections 58.190 and 58.195 of the Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (1987), all records, data and reports will be maintained in storage for a minimum of two years; biological samples will be maintained for a minimum of two years or for as long as the quality of the preparation affords evaluation, whichever is less. These parameters also meet the request of the sponsor that the storage of records be in accordance with EPA Good Laboratory Practice Standards 160.195(b3).

RESULTS

All animals survived to the scheduled sacrifice periods, and no gross lesions were observed in the males at sacrifice.

Body Weight

The mean body weight of the Lewisite-treated males did not differ from that of the control group, throughout the study. The mean body weight of all groups increased approximately 30% over the 14-week measurement period (Figure 1; Table 1).

Figure 1. Mean Body Weight, Males

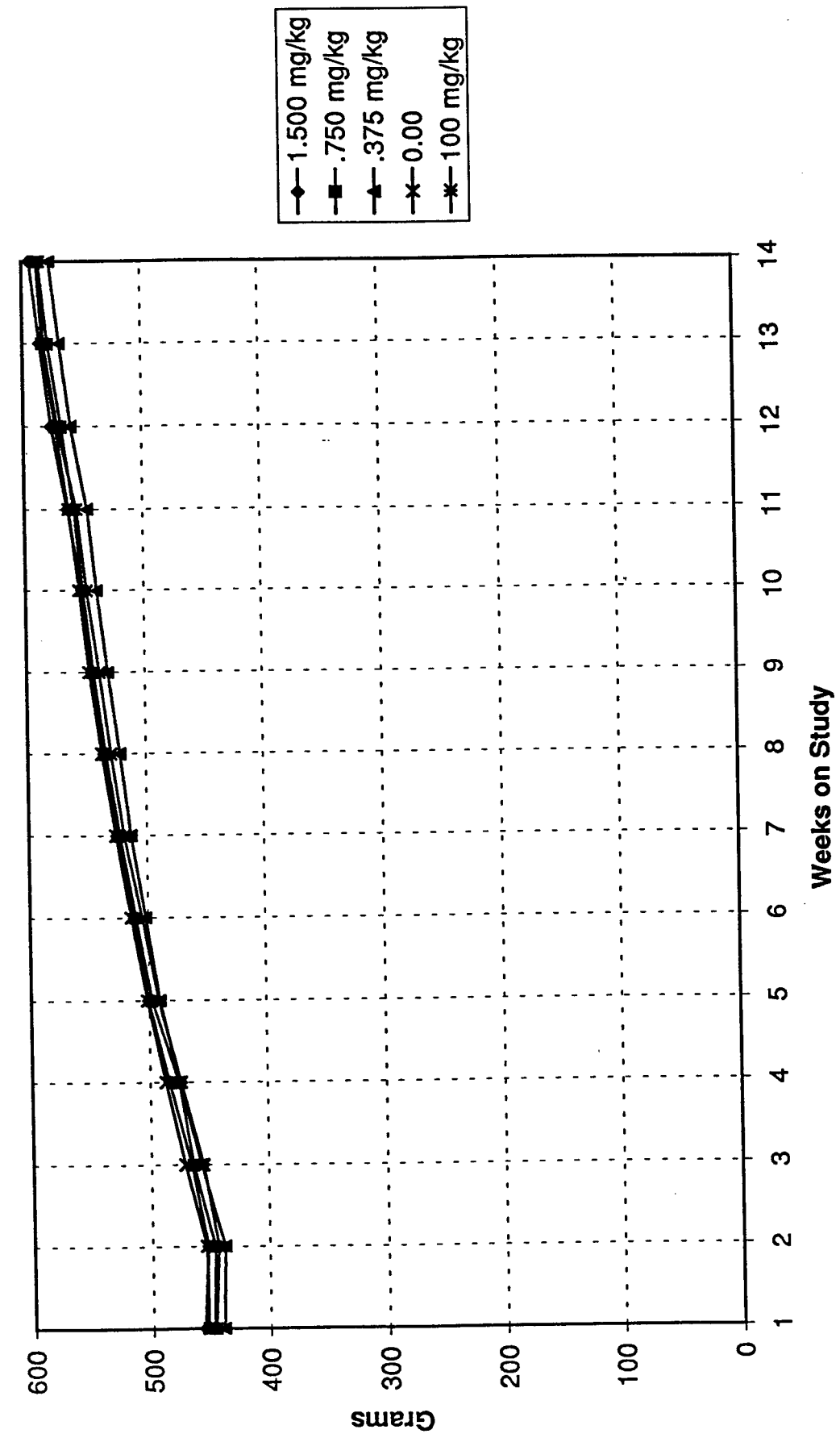

TABLE 1 - WEEKLY MEAN BODY WEIGHTS OF MALE RATS

Male Mean Body, (Grams)					
Week	0.00 mg/kg	0.375 mg/kg	0.750 mg/kg	1.500 mg/kg	EMS 100 mg/kg
1	452.5	439.1	447.6	455.6	446.5
2	453.6	438.1	446.4	452.2	443.5
3	470.9	457.3	463.7	465.8	455.2
4	486.6	475.0	475.3	483.8	473.9
5	501.7	490.7	497.3	500.7	491.8
6	514.7	501.9	510.3	511.9	505.2
7	526.4	513.4	522.8	525.7	519.6
8	537.2	522.1	534.4	535.6	529.5
9	547.3	531.6	543.4	545.3	538.7
10	554.7	539.9	551.5	552.5	548.3
11	562.8	547.0	558.3	563.9	556.3
12	573.3	560.1	568.2	576.5	571.4
13	583.6	568.9	578.8	585.5	582.5
14	588.0	577.1	586.2	593.9	585.8

Reproductive Measures

The endpoints of reproductive performance that were scored were number and percent of mated females that were impregnated, the number and percent (per litter) of total implants, live and dead fetuses, and early, late and total resorptions. The average value per litter for each measure within male treatment group, for each of the 10 weeks of the mating trials, is tabulated in Appendix 1, "Reproductive Measures for the Male Dominant Lethal Study, Weeks 1-10."

Female rats mated to Lewisite-treated males had reproductive performance that was not different from females mated to untreated control males. No effects on reproduction were demonstrated. In contrast, female rats mated to EMS-treated (positive control) males had significantly increased early resorption, consistent with the action of EMS to produce dominant lethal mutations in males. The early resorptions were also reflected as increased total resorptions, decreased live fetuses and decreased total implants (Appendix I, Week 3).

Sperm Motility and Morphology

There were no adverse effects of exposure to Lewisite on motility or morphology of sperm in any of the treated groups, or in the EMS treated group at the time of evaluation (end of the week of cohabitation, Appendix II). Sperm motility by the technique used should have been 75% or greater; the reason for the lower motility recorded in this study (controls included) was not determined. Nevertheless, the rate of impregnation was normal for each group (65-87%) and for this reason, the low motility was suspected to be artefact. The slight increase in sperm motility in the 1.500 mg/kg Lewisite group was significant statistically ($p = 0.028$), but this was not considered to be biologically meaningful. The average values for the respective treatment groups are summarized in Table 2; the individual animal data are included in Appendix II "Sperm Motility and Morphology".

Testicular Weight and Morphometry

There was no difference between the weight of testes from Lewisite-treated males and those of untreated controls. The testes weights were compared as the ratio of combined weight of both testes to the animal's brain weight. The mean value for this relative testicular weight is summarized in Table 2, together with the mean diameter of seminiferous tubules, per treatment group. There was no treatment effect on either of these parameters. Comparison of mean diameter of 200 seminiferous tubules from all rats in the 0.00 and 1.500 mg/kg groups and the EMS group revealed no statistical significant differences. One testes from each animal was examined for histopathologic changes; no morphologic abnormalities were detected. The pathology report and the individual organ weight and morphometric data are included in Appendix III, "Pathology Report, Organ Weight and Testicular Morphometry".

TABLE 2. SUMMARY OF SPERM AND TESTICULAR MEASURES (Mean±SD)

	LEWISITE				ethyl-methane sulfonate
	0.00	0.375 mg/kg	0.750 mg/kg	1.500 mg/kg	100 mg/kg
Sperm Motility (%)	44.4±11.8	41.7±13.3	46.6±13.2	47.0±12.1	45.5±11.2
Abnormal Sperm (%)	1.04	0.81	0.88	0.66	0.70
Sperm Count (10^6/gm cauda)	207.2±36.47	206.3±43.30	212.2±41.23	195.9±34.58	205.1±37.88
Testes/Brain Wt Ratio (%)	1.64±.10	1.64±.15	1.61±.13	1.63±.16	1.67±.08
Diameter, Seminiferous Tubules (μ)	297.3±30.9	not measured	not measured	293.8±27.7	297.3±32.2

SUMMARY AND CONCLUSION

There was no indication of a dominant lethal mutagenic or other toxic effect on the male reproductive tract as a result of exposure to Lewisite, under the conditions of this study. The No Observable Adverse Effect Level in this study was the highest dose used, 1.500 mg/kg.

PERSONNEL

Principal Investigator: Thomas J. Bucci, VMD, PhD.
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Surety Facility Manager: Paul Gosnell
Safety Officer: C. Partridge, T. Cole
Custodians for Agent L: J.A. Crowell, P. Gosnell and R.M. Parker
Statistician: David Gaylor, PhD.
The Biónetics Corporation Personnel: Bob Harmon, Supervisor
Elijah Smith, Technical Specialist

CARE AND USE OF LABORATORY ANIMALS

This protocol will be conducted in accordance to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 85-23, Revised 1985).

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Appendix I: Reproductive Measures for the Male Dominant Lethal Study, Weeks 1-10

TABLE 1 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure Week 1	LEWISITE				
	0.00 (PURPLE)	0.375 MG/KG (YELLOW)	0.750 MG/KG (GREEN)	1.500 MG/KG (RED)	100 MG/KG (BLUE)
Number of Males	20	20	20	20	20
Pregnant Females					
Number	27	28	26	33	30
Percent	67.5	70.0	65.0	82.5	75.0
Number per Litter					
Total Implants	12.8±4.67	14.5±4.14	14.1±4.07	14.4±3.36	15.0±3.05
Live Fetuses	11.3±5.16	13.7±4.31	12.8±4.45	13.4±3.36	12.0±3.52
Dead Fetuses	0.2±0.79	0.1±0.45	0.2±0.51	0.1±0.29	0.2±0.76
Total Resorptions	1.3	0.6	1.0	0.9	2.7
Early Resorptions	0.7±1.11	0.60±0.99	0.9±1.44	0.8±0.78	2.6±2.09
Late Resorptions	0.6±2.88	0.0±0.19	0.1±0.33	0.1±0.29	0.1±0.35
Percent per Litter					
Live Fetuses	88.3	94.4	90.8	93.1	80.0
Dead Fetuses	1.6	0.7	1.4	0.7	1.3
Total Resorptions	10.1	4.1	7.1	6.2	18.0
Early Resorptions	5.5	4.1	6.4	5.6	17.3
Late Resorptions	4.7	0.0	0.7	0.7	0.7
Number of Litters That Had					
Live Fetuses	25	28	26	33	30
Dead Fetuses	2	3	5	3	3
Early Resorptions	11	11	14	20	23
Late Resorptions	3	1	3	3	4
Preimplantation Losses	24	22	21	26	28
Percentage of Litters That Had					
Live Fetuses	93	100	100	100	100
Dead Fetuses	8	11	19	9	10
Early Resorptions	41	39	54	61	77
Late Resorptions	11	4	12	9	13
Preimplantation Losses	89	79	81	79	93

TABLE 2 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure Week 2	LEWISITE				
	0.00 (PURPLE)	0.375 MG/KG (YELLOW)	0.750 MG/KG (GREEN)	1.500 MG/KG (RED)	100 MG/KG (BLUE)
Number of Males	20	20	20	20	20
Pregnant Females					
Number	30	31	27	35	31
Percent	75.0	77.5	69.2	87.5	77.5
Number per Litter					
Total Implants	14.0±3.82	13.7±4.00	15.4±3.02	14.3±3.44	13.5±3.63
Live Fetuses	12.8±4.02	12.9±4.32	13.6±4.04	12.8±3.68	6.6±2.81
Dead Fetuses	0.1±0.25	0.0±0.18	0.2±0.48	0.1±0.40	0.1±0.34
Total Resorptions	1.1	0.8	1.7	1.4	6.8
Early Resorptions	1.1±1.31	0.60±0.84	1.1±1.24	1.1±1 24	6 7±2 63
Late Resorptions	0.0±0.18	0.2±0.73	0.6±2.88	0.3±0.85	0.1±0.34
Percent per Litter					
Live Fetuses	91.4	94.2	88.3	89.5	48.9
Dead Fetuses	0.7	0.0	1.3	0.7	0 7
Total Resorptions	7.8	5.8	11.0	9.8	50.4
Early Resorptions	7.8	4.4	7.1	7.7	49.6
Late Resorptions	0.0	1.5	3.9	2.1	0.7
Number of Litters That Had					
Live Fetuses	30	31	26	34	30
Dead Fetuses	2	1	4	3	4
Early Resorptions	15	14	15	21	31
Late Resorptions	1	2	3	4	4
Preimplantation Losses	25	23	21	27	28
Percentage of Litters That Had					
Live Fetuses	100	100	96	97	97
Dead Fetuses	7	3	15	9	13
Early Resorptions	50	45	56	60	100
Late Resorptions	3	6	11	11	13
Preimplantation Losses	83	74	78	77	90

TABLE 3 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure Week 3	LEWISITE				
	0.00 (PURPLE)	0.375 MG/KG (YELLOW)	0.750 MG/KG (GREEN)	1.500 MG/KG (RED)	100 MG/KG (BLUE)
Number of Males	20	20	20	20	20
Pregnant Females					
Number	31	28	29	31	33
Percent	77.5	70.0	72.5	77.5	82.5
Number per Litter					
Total Implants	14.0±2.93	13.9±2.81	12.7±4.77	14.1±2.53	11.4±3.52
Live Fetuses	12.6±3.64	12.7±2.93	11.1±4.43	12.1±3.54	3.0±2.02
Dead Fetuses	0.1±0.43	0.1±0.26	0.0±0.00	0.7±1.40	0.0±0.17
Total Resorptions	1.3	1.1	1.6	1.3	8.4
Early Resorptions	1.2±1.53	1.1±1.20	1.6±1.40	1.3±1.51	8.3±2.58
Late Resorptions	0.1±0.34	0.0±0.19	0.0±0.19	0.0±0.00	0.1±0.29
Percent per Litter					
Live Fetuses	90.0	91.4	87.4	85.8	26.3
Dead Fetuses	0.7	0.7	0.0	5.0	0.0
Total Resorptions	9.3	7.9	12.6	9.2	73.7
Early Resorptions	8.6	7.9	12.6	9.2	72.8
Late Resorptions	0.7	0.0	0.0	0.0	0.9
Number of Litters That Had					
Live Fetuses	30	28	28	31	29
Dead Fetuses	3	2	0	9	1
Early Resorptions	16	16	22	19	33
Late Resorptions	4	1	1	0	3
Preimplantation Losses	9	24	23	9	27
Percentage of Litters That Had					
Live Fetuses	97	100	97	100	88
Dead Fetuses	10	7	0	29	3
Early Resorptions	52	57	76	61	100
Late Resorptions	13	4	3	0	9
Preimplantation Losses	29	86	79	29	82

TABLE 4 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure Week 4	LEWISITE				
	0.00 (PURPLE)	0.375 MG/KG (YELLOW)	0.750 MG/KG (GREEN)	1.500 MG/KG (RED)	100 MG/KG (BLUE)
Number of Males	20	20	20	20	20
Pregnant Females					
Number	35	26	30	33	32
Percent	87.5	65.0	75.0	82.5	80.0
Number per Litter					
Total Implants	15.0±3.61	15.4±2.30	14.1±4.19	16.4±2.07	15.2±2.94
Live Fetuses	13.1±3.23	13.8±2.52	13.0±4.07	14.7±2.52	11.8±3.65
Dead Fetuses	0.2±0.51	0.2±0.51	0.1±0.35	0.1±0.38	0.2±0.54
Total Resorptions	1.7	1.3	1.0	1.5	3.3
Early Resorptions	1.7±1.71	1.3±1.52	1.0±1.16	1.5±1.66	3.2±2.77
Late Resorptions	0.0±0.17	0.0±0.20	0.0±0.00	0.0±0.17	0.1±0.34
Percent per Litter					
Live Fetuses	87.3	89.6	92.2	89.6	77.6
Dead Fetuses	1.3	1.3	0.7	0.6	1.3
Total Resorptions	11.3	8.4	7.1	9.1	21.7
Early Resorptions	11.3	8.4	7.1	9.1	21.1
Late Resorptions	0.0	0.0	0.0	0.0	0.6
Number of Litters That Had					
Live Fetuses	35	26	29	33	32
Dead Fetuses	4	5	4	2	4
Early Resorptions	26	19	17	25	27
Late Resorptions	1	1	0	1	4
Preimplantation Losses	29	22	24	27	24
Percentage of Litters That Had					
Live Fetuses	100	100	97	100	100
Dead Fetuses	11	19	13	6	13
Early Resorptions	74	73	57	76	84
Late Resorptions	3	4	0	3	13
Preimplantation Losses	83	85	80	82	75

TABLE 5 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure **Week 5**	LEWISITE				
	0.00 (PURPLE)	**0.375 MG/KG (YELLOW)**	**0.750 MG/KG (GREEN)**	**1.500 MG/KG (RED)**	**100 MG/KG (BLUE)**
Number of Males	20	20	20	20	20
Pregnant Females					
Number	31	30	32	33	32
Percent	77.5	75.0	80.0	82.5	80.0
Number per Litter					
Total Implants	14.2±3.95	13.5±4.95	14.0±5.10	15.1±3.58	14.6±3.89
Live Fetuses	12.7±4.21	11.9±5.53	12.8±5.07	13.2±4.61	13.1±4.32
Dead Fetuses	0.1±0.25	0.0±0.18	0.1±0.25	0.1±0.24	0.1±0.30
Total Resorptions	1.4	1.5	1.2	1.8	1.5
Early Resorptions	1.2±1.41	1.5±1.78	1.2±1.25	1.5±1.82	1.5±1.78
Late Resorptions	0.2±0.40	0.0±0.18	0.0±0.18	0.3±1.74	0.0±0.00
Percent per Litter					
Live Fetuses	89.4	88.1	91.4	87.4	89.7
Dead Fetuses	0.7	0.0	0.7	0.6	0.7
Total Resorptions	9.9	11.1	8.6	11.9	10.3
Early Resorptions	8.5	11.1	8.6	9.9	10.3
Late Resorptions	1.4	0.0	0.0	2.0	0.0
Number of Litters That Had					
Live Fetuses	30	27	31	32	32
Dead Fetuses	2	1	2	2	3
Early Resorptions	20	21	21	21	20
Late Resorptions	6	1	1	2	0
Preimplantation Losses	25	25	26	20	23
Percentage of Litters That Had					
Live Fetuses	97	90	97	97	100
Dead Fetuses	6	3	6	6	9
Early Resorptions	65	70	66	64	63
Late Resorptions	19	3	3	6	0
Preimplantation Losses	81	83	81	58	72

TABLE 6 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure **Week 6**	LEWISITE				
	0.00 (PURPLE)	**0.375 MG/KG (YELLOW)**	**0.750 MG/KG (GREEN)**	**1.500 MG/KG (RED)**	**100 MG/KG (BLUE)**
Number of Males	20	20	20	20	20
Pregnant Females					
Number	32	33	27	35	33
Percent	80.0	82.5	67.5	87.5	82.5
Number per Litter					
Total Implants	12.9±5.51	15:2±2.33	11.7±4.78	14.3±3.84	14.8±3.51
Live Fetuses	11.8±5.38	13.5±3.21	10.3±4.71	12.7±4.08	13.4±3.73
Dead Fetuses	0.1±0.42	0.2±0.60	0.0±0.19	0.1±0.24	0.1±0.24
Total Resorptions	0.9	1.6	1.3	1.6	1.4
Early Resorptions	0.9±1.11	1.5±1.64	0.9±0.83	1.5±2.32	1.3±1.21
Late Resorptions	0.0±0.18	0.1±0.33	0.4±1.31	0.1±0.24	0.1±0.24
Percent per Litter					
Live Fetuses	91.5	88.8	88.0	88.8	90.5
Dead Fetuses	0.8	1.3	0.0	0.7	0.7
Total Resorptions	7.0	10.5	12.6	11.2	9.5
Early Resorptions	7.0	9.9	8.7	10.5	8.8
Late Resorptions	0.0	0.7	3.9	0.7	0.7
Number of Litters That Had					
Live Fetuses	32	33	26	35	33
Dead Fetuses	3	5	1	2	2
Early Resorptions	19	23	18	25	23
Late Resorptions	1	4	4	2	2
Preimplantation Losses	27	26	22	24	22
Percentage of Litters That Had					
Live Fetuses	100	100	96	100	100
Dead Fetuses	9	15	4	6	6
Early Resorptions	59	70	67	71	70
Late Resorptions	3	17	15	6	6
Preimplantation Losses	84	79	81	69	67

TABLE 7 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure Week 7	LEWISITE				
	0.00 (PURPLE)	0.375 MG/KG (YELLOW)	0.750 MG/KG (GREEN)	1.500 MG/KG (RED)	100 MG/KG (BLUE)
Number of Males	20	20	20	20	20
Pregnant Females					
- Number	31	28	28	33	30
- Percent	77.5	70.0	70.0	82.5	75.0
Number per Litter					
Total Implants	14.2±3.49	13.3±4.12	13.5±3.81	15.3±1.90	15.2±2.14
Live Fetuses	12.9±3.81	12.3±4.13	12.1±3.62	13.9±2.32	13.6±3.08
Dead Fetuses	0.2±0.48	0.0±0.19	0.3±0.65	0.1±0.38	0.3±0.84
Total Resorptions	1.2	1.0	1.1	1.3	1.3
Early Resorptions	1.1±1.36	0.9±0.88	1.0±1.22	1.3±1.38	1.1±0.98
Late Resorptions	0.1±0.25	0.1±0.26	0.1±0.52	0.0±0.17	0.2±0.57
Percent per Litter					
Live Fetuses	90.2	92.5	89.6	90.8	89.5
Dead Fetuses	1.4	0.0	2.2	0.7	2.0
Total Resorptions	8.4	75.2	8.1	8.5	8.6
Early Resorptions	7.7	67.7	7.4	8.5	7.2
Late Resorptions	0.7	0.8	0.7	0.0	1.3
Number of Litters That Had					
Live Fetuses	31	28	28	33	30
Dead Fetuses	5	1	4	2	5
Early Resorptions	20	16	14	22	22
Late Resorptions	2	2	2	1	5
Preimplantation Losses	25	22	22	21	26
Percentage of Litters That Had					
Live Fetuses	100	100	100	100	100
Dead Fetuses	16	4	14	6	17
Early Resorptions	65	57	50	67	73
Late Resorptions	6	7	7	3	17
Preimplantation Losses	81	79	79	64	87

TABLE 8 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure **Week 8**	LEWISITE				
	0.00 (PURPLE)	**0.375 MG/KG (YELLOW)**	**0.750 MG/KG (GREEN)**	**1.500 MG/KG (RED)**	**100 MG/KG (BLUE)**
Number of Males	20	20	20	20	20
Pregnant Females					
- Number	30	28	28	32	31
- Percent	75.0	70.0	70.0	80.0	77.5
Number per Litter					
Total Implants	13.0±4.50	14.0±4.10	13.0±3.94	14.4±2.47	14.6±3.80
Live Fetuses	11.9±4.53	12.4±3.97	11.7±3.94	13.0±3.43	13.4±4.08
Dead Fetuses	0.0±0.18	0.1±0.38	0.1±0.26	0.0±0.18	0.0±0.00
Total Resorptions	1.0	1.6	1.2	1.4	1.2
Early Resorptions	1.0±1.26	1.3±1.42	1.2±1.31	1.3±1.91	1.1±1.09
Late Resorptions	0.0±0.00	0.3±1.14	0.0±0.19	0.1±0.25	0.1±0.25
Percent per Litter					
Live Fetuses	91.5	88.6	90.0	90.3	91.8
Dead Fetuses	0.0	0.7	0.8	0.0	0.0
Total Resorptions	7.7	11.4	9.2	9.7	8.2
Early Resorptions	7.7	9.3	9.2	9.0	7.5
Late Resorptions	0.0	2.1	0.0	0.7	0.7
Number of Litters That Had					
Live Fetuses	30	28	28	32	31
Dead Fetuses	1	1	2	1	0
Early Resorptions	17	16	18	20	20
Late Resorptions	0	2	1	2	2
Preimplantation Losses	23	7	8	27	26
Percentage of Litters That Had					
Live Fetuses	100	100	100	100	100
Dead Fetuses	3	4	7	3	0
Early Resorptions	57	57	64	63	65
Late Resorptions	0	7	4	6	6
Preimplantation Losses	77	25	29	84	84

TABLE 9 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure **Week 9**	LEWISITE				
	0.00 (PURPLE)	**0.375 MG/KG (YELLOW)**	**0.750 MG/KG (GREEN)**	**1.500 MG/KG (RED)**	**100 MG/KG (BLUE)**
Number of Males	20	20	20	20	20
Pregnant Females					
Number	28	31	29	27	33
Percent	70.0	77.5	72.5	67.5	82.5
Number per Litter					
Total Implants	14.5±3.88	14.1±3.66	13.5±4.71	14.9±2.44	14.2±3.03
Live Fetuses	12.6±4.08	12.6±4.75	12.0±4.79	13.6±2.62	13.2±3.19
Dead Fetuses	0.1±0.42	0.1±0.30	0.0±0.19	0.0±0.00	0.0±0.00
Total Resorptions	1.7	1.4	1.5	1.2	1.0
Early Resorptions	1.7±1.30	1.4±1.80	1.5±2.15	1.2±1.22	0.9±0.86
Late Resorptions	0.0±0.00	0.0±0.18	0.0±0.19	0.0±0.00	0.1±0.24
Percent per Litter					
Live Fetuses	86.9	89.4	88.9	91.3	93.0
Dead Fetuses	0.7	0.7	0.0	0.0	0.0
Total Resorptions	11.8	9.9	11.1	8.1	7.0
Early Resorptions	11.8	9.9	11.1	8.1	6.3
Late Resorptions	0.0	0.0	0.0	0.0	0.7
Number of Litters That Had					
Live Fetuses	28	30	29	27	33
Dead Fetuses	2	3	1	0	0
Early Resorptions	22	21	15	20	21
Late Resorptions	0	1	1	0	2
Preimplantation Losses	20	23	22	23	28
Percentage of Litters That Had					
Live Fetuses	100	97	100	100	100
Dead Fetuses	7	10	3	0	0
Early Resorptions	79	68	52	74	64
Late Resorptions	0	3	3	0	6
Preimplantation Losses	71	74	76	85	85

TABLE 10 REPRODUCTIVE MEASURES FOR THE MALE DOMINANT STUDY

Post-Exposure Week 10	LEWISITE				
	0.00 (PURPLE)	0.375 MG/KG (YELLOW)	0.750 MG/KG (GREEN)	1.500 MG/KG (RED)	100 MG/KG (BLUE)
Number of Males	20	20	20	20	20
Pregnant Females					
- Number	29	27	31	31	26
- Percent	72.5	67.5	77.5	77.5	65.0
Number per Litter					
Total Implants	12.6±4.87	13.6±4.80	13.9±3.92	14.8±3.03	14.0±4.80
Live Fetuses	11.3±4.87	12.7±4.49	13.0±3.98	13.4±3.44	13.0±4.96
Dead Fetuses	0.0±0.19	0.1±0.27	0.0±0.00	0.1±0.25	0.0±0.00
Total Resorptions	1.1	0.8	0.9	1.3	1.0
Early Resorptions	1.0±1.09	0.7±0.73	0.8±1.14	1.3±1.19	1.0±0.96
Late Resorptions	0.1±0.35	0.1±0.58	0.1±0.40	0.0±0.00	0.0±0.00
Percent per Litter					
Live Fetuses	89.7	93.3	93.5	90.5	92.9
Dead Fetuses	0.0	0.7	0.0	0.7	0.0
Total Resorptions	8.7	5.9	6.5	8.8	7.1
Early Resorptions	7.9	5.1	5.8	8.8	7.1
Late Resorptions	0.8	0.7	7.2	0.0	0.0
Number of Litters That Had					
Live Fetuses	29	27	31	31	25
Dead Fetuses	1	2	0	2	0
Early Resorptions	18	14	14	21	17
Late Resorptions	4	1	2	0	0
Preimplantation Losses	22	23	27	28	23
Percentage of Litters That Had					
Live Fetuses	100	100	100	100	96
Dead Fetuses	3	7	0	6	0
Early Resorptions	62	52	45	68	65
Late Resorptions	14	4	6	0	0
Preimplantation Losses	76	85	87	90	88

Appendix II: Sperm Motility and Morphology

DOMINANT LETHAL - LEWISITE : SPERM MOTILITY DATA										
ANIMAL #	DOSE MG/KG	CID	SIDE	ACC #	DATE	# CELLS	# MOT.	# NON-MOT	CONC.	%MOTILE
5	0.00	2011	L	9	4/9/90	214	56	158	263.570	26.17
5	0.00	2011	R	10	4/9/90	236	100	136	228.878	42.37
9	0.00	2015	L	17	4/9/90	200	59	141	248.928	29.50
9	0.00	2015	R	18	4/9/90	210	64	146	204.549	30.48
13	0.00	2019	L	25	4/9/90	ND				
13	0.00	2019	R	26	4/9/90	ND				
17	0.00	2023	L	33	4/9/90	217	53	164	224.373	24.42
17	0.00	2023	R	34	4/9/90	202	58	144	204.549	28.71
25	0.00	2031	L	9	4/10/90	222	128	94	268.076	57.66
25	0.00	2031	R	10	4/10/90	209	83	126	176.465	39.71
29	0.00	2035	L	17	4/10/90	211	63	148	206.351	29.86
29	0.00	2035	R	18	4/10/90	205	111	94	208.153	54.15
33	0.00	2039	L	25	4/10/90	243	102	141	198.992	41.98
33	0.00	2039	R	26	4/10/90	211	82	129	180.219	38.86
37	0.00	2043	L	33	4/10/90	203	133	70	193.736	65.52
37	0.00	2043	R	34	4/10/90	222	120	102	175.714	54.05
45	0.00	2051	L	9	4/11/90	220	99	121	223.472	45.00
45	0.00	2051	R	10	4/11/90	208	116	92	260.191	55.77
49	0.00	2055	L	17	4/11/90	218	140	78	182.472	64.22
49	0.00	2055	R	18	4/11/90	248	173	75	239.691	69.76
53	0.00	2059	L	25	4/11/90	224	109	115	162.197	48.66
53	0.00	2059	R	26	4/11/90	224	134	90	186.226	59.82
57	0.00	2062	L	31	4/11/90	209	81	128	220.768	38.76
57	0.00	2062	R	32	4/11/90	219	82	137	167.346	37.44
65	0.00	2070	L	9	4/12/90	257	94	163	172.335	36.58
65	0.00	2070	R	10	4/12/90	204	67	137	186.977	32.84
69	0.00	2074	L	17	4/12/90	210	84	126	222.571	40.00
69	0.00	2074	R	18	4/12/90	219	75	143	227.977	34.70
73	0.00	2078	L	25	4/12/90	204	92	112	178.717	45.10
73	0.00	2078	R	26	4/12/90	223	112	111	231.582	50.22
77	0.00	2082	L	33	4/12/90	217	114	103	227.977	52.53
77	0.00	2082	R	34	4/12/90	234	153	81	239.691	65.38
85	0.00	2090	L	9	4/13/90	203	77	126	254.560	37.93
85	0.00	2090	R	10	4/13/90	234	88	146	237.889	37.61
89	0.00	2094	L	17	4/13/90	227	97	130	140.233	42.73
89	0.00	2094	R	18	4/13/90	227	94	133	227.075	41.41
93	0.00	2098	L	25	4/13/90	201	95	106	118.144	47.26
93	0.00	2098	R	26	4/13/90	206	113	93	134.038	54.80
97	0.00	2101	L	31	4/13/90	204	75	129	202.747	36.76
97	0.00	2101	R	32	4/13/90	221	117	124	244.197	48.55
						217.53	97.18	120.84	207.15	44.40
								STDEV	36.47	

DOMINANT LETHAL - LEWISITE : SPERM MOTILITY DATA										
ANIMAL #	DOSE MG/KG	CID	SIDE	ACC #	DATE	# CELLS	# MOT.	# NON-MOT	CONC.	%MOTILE
3	0.375	2009	L	5	4/9/90	229	42	187	186.226	18.34
3	0.375	2009	R	6	4/9/90	219	48	171	215.362	21.92
7	0.375	2013	L	13	4/9/90	228	52	176	231.582	22.81
7	0.375	2013	R	14	4/9/90	222	79	143	225.274	35.59
11	0.375	2017	L	21	4/9/90	229	70	159	233.384	30.57
11	0.375	2017	R	22	4/9/90	229	90	139	231.582	39.30
20	0.375	2026	L	39	4/9/90	201	66	135	200.043	32.84
20	0.375	2026	L	40	4/9/90	203	51	152	214.461	25.12
23	0.375	2029	L	5	4/10/90	205	153	52	243.296	74.63
23	0.375	2029	R	6	4/10/90	203	104	99	250.054	51.23
27	0.375	2033	L	13	4/10/90	218	100	118	208.153	45.87
27	0.375	2033	R	14	4/10/90	203	82	121	200.944	40.39
31	0.375	2037	L	21	4/10/90	226	77	149	221.670	34.07
31	0.375	2037	R	22	4/10/90	215	88	127	183.974	40.93
40	0.375	2046	L	39	4/10/90	224	93	131	224.373	41.52
40	0.375	2046	L	40	4/10/90	239	71	168	193.736	29.71
43	0.375	2049	L	5	4/11/90	226	130	96	163.485	57.52
43	0.375	2049	R	6	4/11/90	232	162	70	231.582	69.83
47	0.375	2053	L	13	4/11/90	248	100	148	255.010	40.32
47	0.375	2053	R	14	4/11/90	231	121	110	191.483	52.38
51	0.375	2057	L	21	4/11/90	243	110	133	201.245	45.27
51	0.375	2057	R	22	4/11/90	209	62	147	175.714	29.67
60	0.375	2065	L	37	4/11/90	230	87	143	189.230	37.83
60	0.375	2065	R	38	4/11/90	238	113	125	231.582	47.48
63	0.375	2068	L	5	4/12/90	251	145	106	213.259	57.77
63	0.375	2068	R	6	4/12/90	209	75	134	263.570	35.89
67	0.375	2072	L	13	4/12/90	208	109	99	129.157	52.40
67	0.375	2072	R	14	4/12/90	200	86	114	110.384	43.00
71	0.375	2076	L	21	4/12/90	202	112	90	253.433	55.45
71	0.375	2076	R	22	4/12/90	209	99	110	266.950	47.37
80	0.375	2085	L	39	4/12/90	226	120	106	194.486	53.10
80	0.375	2085	R	40	4/12/90	206	123	83	208.153	59.71
83	0.375	2088	L	5	4/13/90	221	102	119	232.483	46.15
83	0.375	2088	R	6	4/13/90	227	107	120	281.592	47.14
87	0.375	2092	L	13	4/13/90	214	75	139	224.373	35.05
87	0.375	2092	R	14	4/13/90	228	59	169	91.496	25.88
91	0.375	2096	L	21	4/13/90	237	102	135	180.219	43.04
91	0.375	2096	R	22	4/13/90	224	114	110	229.779	50.89
100	0.375	2104	L	37	4/13/90	212	78	134	169.921	36.79
100	0.375	2104	R	38	4/13/90	210	30	180	97.994	14.29
						220.85	92.18	128.68	206.27	41.73
								STDEV	43.30	

DOMINANT LETHAL - LEWISITE : SPERM MOTILITY DATA										
ANIMAL #	DOSE MG/KG	CID	SIDE	ACC #	DATE	# CELLS	# MOT.	# NON-MOT	CONC.	%MOTILE
2	0.750	2008	L	3	4/9/90	ND				
2	0.750	2008	R	4	4/9/90	231	75	156	224.373	32.47
6	0.750	2012	L	11	4/9/90	199	42	157	167.454	21.11
6	0.750	2012	R	12	4/9/90	219	96	123	224.373	43.84
15	0.750	2021	L	29	4/9/90	236	72	164	237.889	30.51
15	0.750	2021	R	30	4/9/90	199	72	127	149.968	36.18
19	0.750	2025	L	37	4/9/90	200	54	146	246.675	27.00
19	0.750	2025	R	38	4/9/90	196	71	125	248.928	36.22
22	0.750	2028	L	3	4/10/90	224	126	98	185.476	56.25
22	0.750	2028	R	4	4/10/90	210	147	63	248.928	70.00
26	0.750	2032	L	11	4/10/90	240	160	80	240.593	66.67
26	0.750	2032	R	12	4/10/90	210	95	115	213.560	45.24
35	0.750	2041	L	29	4/10/90	206	82	124	168.955	39.81
35	0.750	2041	R	30	4/10/90	230	78	152	226.175	33.91
39	0.750	2045	L	37	4/10/90	203	120	83	247.801	59.11
39	0.750	2045	R	38	4/10/90	213	109	104	260.191	51.17
42	0.750	2048	L	3	4/11/90	214	114	100	136.291	53.27
42	0.750	2048	R	4	4/11/90	224	125	99	182.472	55.80
46	0.750	2052	L	11	4/11/90	273	161	112	273.032	58.97
46	0.750	2052	R	12	4/11/90	246	169	77	241.494	68.70
59	0.750	2064	L	35	4/11/90	241	98	143	247.801	40.66
59	0.750	2064	R	36	4/11/90	216	64	152	214.461	29.63
62	0.750	2067	L	3	4/12/90	218	152	66	269.202	69.72
62	0.750	2067	R	4	4/12/90	219	112	107	229.779	51.14
66	0.750	2071	L	11	4/12/90	222	73	149	121.548	32.88
66	0.750	2071	R	12	4/12/90	206	75	131	133.162	36.41
75	0.750	2080	L	29	4/12/90	200	96	104	201.845	48.00
75	0.750	2080	R	30	4/12/90	208	97	111	219.867	46.63
79	0.750	2084	L	37	4/12/90	221	139	82	222.571	62.90
79	0.750	2084	R	38	4/12/90	223	116	107	229.779	52.02
82	0.750	2087	L	3	4/13/90	234	113	121	172.495	48.29
82	0.750	2087	R	4	4/13/90	250	111	139	252.307	44.40
86	0.750	2091	L	11	4/13/90	221	128	93	182.472	57.92
86	0.750	2091	R	12	4/13/90	ND				
99	0.750	2103	L	35	4/13/90	215	65	150	160.415	30.28
99	0.750	2103	R	36	4/13/90	227	108	119	231.582	47.58
						336.05	104.68	115.96	212.17	47.37
								STDEV	41.23	

DOMINANT LETHAL - LEWISITE : SPERM MOTILITY DATA										
ANIMAL #	DOSE MG/KG	CID	SIDE	ACC #	DATE	# CELLS	# MOT.	# NON-MOT	CONC.	%MOTILE
1	1.500	2007	L	1	4/9/90	211	63	148	206.351	29.86
1	1.500	2007	R	2	4/9/90	ND				
10	1.500	2016	L	19	4/9/90	193	38	155	164.450	19.69
10	1.500	2016	R	20	4/9/90	221	89	132	281.592	40.27
14	1.500	2020	L	27	4/9/90	ND				
14	1.500	2020	R	28	4/9/90	196	66	130	200.944	33.67
18	1.500	2024	L	35	4/9/90	200	64	136	203.648	32.00
18	1.500	2024	R	36	4/9/90	230	94	136	192.985	40.87
21	1.500	2027	L	1	4/10/90	194	74	120	124.464	38.14
21	1.500	2027	R	2	4/10/90	202	94	108	146.750	46.53
30	1.500	2036	L	19	4/10/90	209	87	122	148.037	41.63
30	1.500	2036	R	20	4/10/90	228	132	96	222.571	57.89
34	1.500	2040	L	27	4/10/90	241	101	140	196.739	41.91
34	1.500	2040	R	28	4/10/90	217	86	131	189.230	39.63
38	1.500	2044	L	35	4/10/90	214	100	114	215.362	46.73
38	1.500	2044	R	36	4/10/90	240	129	111	239.691	53.75
41	1.500	2047	L	1	4/11/90	242	184	58	237.889	76.03
41	1.500	2047	R	2	4/11/90	215	123	92	186.226	57.21
50	1.500	2056	L	19	4/11/90	ND				
50	1.500	2056	R	20	4/11/90	244	145	99	246.900	59.43
54	1.500	2060	L	27	4/11/90	225	158	67	224.373	70.22
54	1.500	2060	R	28	4/11/90	223	121	102	184.725	54.26
58	1.500	2063	L	33	4/11/90	231	143	88	226.175	61.90
58	1.500	2063	R	34	4/11/90	217	100	117	207.252	46.08
61	1.500	2066	L	1	4/12/90	23	92	141	200.494	39.48
61	1.500	2066	R	2	4/12/90	214	106	108	140.796	49.53
70	1.500	2075	L	19	4/12/90	207	111	96	255.686	53.62
70	1.500	2075	R	20	4/12/90	214	105	109	218.966	49.07
74	1.500	2079	L	27	4/12/90	220	123	97	159.623	55.91
74	1.500	2079	R	28	4/12/90	216	119	97	182.472	55.09
78	1.500	2083	L	35	4/12/90	208	114	94	179.468	54.81
78	1.500	2083	R	36	4/12/90	249	168	81	207.252	67.47
81	1.500	2086	L	1	4/13/90	228	99	129	172.495	43.42
81	1.500	2086	R	2	4/13/90	234	80	154	196.739	34.19
90	1.500	2095	L	19	4/13/90	210	84	126	181.721	40.00
90	1.500	2095	R	20	4/13/90	224	107	117	146.428	47.77
94	1.500	2099	L	27	4/13/90	200	101	99	173.461	50.50
94	1.500	2099	R	28	4/13/90	217	116	101	219.867	53.46
98	1.500	2102	L	33	4/13/90	220	64	156	169.921	29.09
98	1.500	2102	R	34	4/13/90	ND				
						213.25	105.00	114.08	195.88	47.53
								STDEV	34.58	

DOMINANT LETHAL - EMS : SPERM MOTILITY DATA										
ANIMAL #	DOSE MG/KG	CID	SIDE	ACC #	DATE	# CELLS	# MOT.	# NON-MOT	CONC.	%MOTILE
4	100	2010	L	7	4/9/90	157	54	103	193.736	34.39
4	100	2010	R	8	4/9/90	201	58	143	203.648	28.86
8	100	2014	L	15	4/9/90	224	50	174	224.373	22.32
8	100	2014	R	16	4/9/90	200	61	139	172.710	30.50
12	100	2018	L	23	4/9/90	242	87	155	241.494	350.95
12	100	2018	R	24	4/9/90	228	83	145	190.732	36.40
16	100	2022	L	31	4/9/90	249	57	192	252.307	22.89
16	100	2022	L	32	4/9/90	220	85	135	223.472	38.64
24	100	2030	L	7	4/10/90	201	79	122	239.917	39.30
24	100	2030	R	8	4/10/90	222	134	88	223.472	60.36
28	100	2034	L	15	4/10/90	225	118	107	222.571	52.44
28	100	2034	R	16	4/10/90	201	79	122	163.699	39.30
32	100	2038	L	23	4/10/90	ND				
32	100	2038	R	24	4/10/90	211	115	96	256.812	54.50
36	100	2042	L	31	4/10/90	199	76	123	193.736	38.19
36	100	2042	R	32	4/10/90	212	95	117	183.223	44.81
44	100	2050	L	7	4/11/90	206	130	76	199.142	63.11
44	100	2050	R	8	4/11/90	215	147	68	215.362	68.37
48	100	2054	L	15	4/11/90	ND				
48	100	2054	R	16	4/11/90	239	104	135	201.996	43.51
52	100	2058	L	23	4/11/90	238	107	131	232.483	44.96
52	100	2058	R	24	4/11/90	239	96	143	189.981	40.17
56	100	2061	L	29	4/11/90	203	155	88	168.205	56.65
56	100	2061	R	30	4/11/90	214	111	103	163.485	51.87
64	100	2069	L	7	4/12/90	206	115	91	177.966	55.83
64	100	2069	R	8	4/12/90	213	96	117	148.118	45.07
68	100	2073	L	15	4/12/90	222	109	113	93.229	49.10
68	100	2073	R	16	4/12/90	211	112	99	262.444	53.08
72	100	2077	L	23	4/12/90	214	91	123	177.215	42.52
72	100	2077	R	24	4/12/90	213	101	112	270.329	47.42
76	100	2081	L	31	4/12/90	217	91	126	226.175	41.94
76	100	2081	R	32	4/12/90	238	141	97	246.900	59.24
84	100	2089	L	7	4/13/90	222	105	117	219.867	47.30
84	100	2089	R	8	4/13/90	211	77	134	264.597	36.49
88	100	2093	L	15	4/13/90	173	76	97	225.274	43.93
88	100	2093	R	16	4/13/90	229	79	150	159.277	34.50
92	100	2097	L	23	4/13/90	228	150	78	224.373	65.79
92	100	2097	R	24	4/13/90	240	136	104	198.241	56.67
96	100	2100	L	29	4/13/90	224	120	104	187.728	53.57
96	100	2100	R	30	4/13/90	209	107	102	156.404	51.20
						4603.00	99.66	117.61	205.12	53.85
								STDEV	37.88	

SPERM HEAD MORPHOLOGY

MALE	LEWISITE (mg/kg)	NORMAL	NO HOOK	EXCESS. HOOK	AMORPHOUS	PIN-HEAD	TWO HEADS/TAILS	SHORT	OTHER	% ABNORMAL
5	0.00	497	1	0	0	0	2	0	0	0.60%
9	0.00	499	1	0	0	0	0	0	0	0.20%
13	0.00	495	4	1	0	0	0	0	0	1.00%
17	0.00	500	0	0	0	0	0	0	0	0.00%
25	0.00	497	3	0	0	0	0	0	0	0.60%
29	0.00	497	3	0	0	0	0	0	0	0.60%
33	0.00	498	2	0	0	0	0	0	0	0.40%
37	0.00	500	0	0	0	0	0	0	0	0.00%
45	0.00	492	3	0	5	0	0	0	0	1.60%
49	0.00	500	0	0	0	0	0	0	0	0.00%
53	0.00	499	1	0	0	0	0	0	0	0.20%
57	0.00	497	2	0	0	0	1	0	0	0.60%
65	0.00	495	4	0	1	0	0	0	0	1.00%
69	0.00	490	9	0	1	0	0	0	0	2.00%
73	0.00	500	0	0	0	0	0	0	0	0.00%
77	0.00	495	5	0	0	0	0	0	0	1.00%
85	0.00	495	4	1	0	0	0	0	0	1.00%
89	0.00	474	26	0	0	0	0	0	0	5.20%
93	0.00	483	17	0	0	0	0	0	0	3.40%
97	0.00	493	4	3	0	0	0	0	0	1.40%
		494.8	4.45	0.25	0.35	0.00	0.15	0.00	0.00	1.04%

SPERM HEAD MORPHOLOGY

MALE	LEWISITE (mg/kg)	NORMAL	NO HOOK	EXCESS. HOOK	AMORPHOUS	PIN-HEAD	TWO HEADS/TAILS	SHORT	OTHER	% ABNORMAL
3	0.375	498	1	0	1	0	0	0	0	0.40%
7	0.375	500	0	0	0	0	0	0	0	0.00%
11	0.375	499	1	0	0	0	0	0	0	0.20%
20	0.375	499	1	0	0	0	0	0	0	0.20%
23	0.375	499	0	1	0	0	0	0	0	0.20%
27	0.375	497	3	0	0	0	0	0	0	0.60%
31	0.375	500	0	0	0	0	0	0	0	0.00%
40	0.375	492	8	0	0	0	0	0	0	1.60%
43	0.375	498	1	1	0	0	0	0	0	0.40%
47	0.375	497	3	0	0	0	0	0	0	0.60%
51	0.375	497	3	0	0	0	0	0	0	0.60%
60	0.375	500	0	0	0	0	0	0	0	0.00%
63	0.375	486	14	0	0	0	0	0	0	2.80%
67	0.375	484	15	1	0	0	0	0	0	3.20%
71	0.375	500	0	0	0	0	0	0	0	0.00%
80	0.375	499	1	0	0	0	0	0	0	0.20%
83	0.375	490	8	2	0	0	0	0	0	2.00%
87	0.375	494	6	0	0	0	0	0	0	1.20%
91	0.375	493	7	0	0	0	0	0	0	1.40%
100	0.375	497	2	0	1	0	0	0	0	0.60%
		495.95	3.70	0.25	0.10	0.00	0.00	0.00	0.00	0.81%

SPERM HEAD MORPHOLOGY

MALE	LEWISITE (mg/kg)	NORMAL	NO HOOK	EXCESS HOOK	AMORPHOUS	PIN-HEAD	TWO HEADS/TAILS	SHORT	OTHER	% ABNORMAL
2	0.750	498	2	0	0	0	0	0	0	0.40%
6	0.750	498	2	0	0	0	0	0	0	0.40%
15	0.750	500	0	0	0	0	0	0	0	0.00%
19	0.750	500	0	0	0	0	0	0	0	0.00%
22	0.750	498	2	0	0	0	0	0	0	0.40%
26	0.750	498	2	0	0	0	0	0	0	0.40%
35	0.750	498	2	0	0	0	0	0	0	0.40%
39	0.750	499	1	0	0	0	0	0	0	0.20%
42	0.750	500	0	0	0	0	0	0	0	0.00%
46	0.750	493	7	0	0	0	0	0	0	1.40%
59	0.750	497	3	0	0	0	0	0	0	0.60%
62	0.750	499	0	1	0	0	0	0	0	0.20%
66	0.750	489	11	0	0	0	0	0	0	2.20%
75	0.750	497	3	0	0	0	0	0	0	0.60%
79	0.750	497	3	0	0	0	0	0	0	0.60%
82	0.750	487	10	3	0	0	0	0	0	2.60%
86	0.750	487	13	0	0	0	0	0	0	2.60%
99	0.750	486	14	0	0	0	0	0	0	2.80%
		495.61	4.17	0.22	0.00	0.00	0.00	0.00	0.00	0.88%

SPERM HEAD MORPHOLOGY

MALE	LEWISITE (mg/kg)	NORMAL	NO HOOK	EXCESS. HOOK	AMORPHOUS	PIN-HEAD	TWO HEADS/TAILS	SHORT	OTHER	% ABNORMAL
1	1.500	500	0	0	0	0	0	0	0	0.00%
10	1.500	500	0	0	0	0	0	0	0	0.00%
14	1.500	498	2	0	0	0	0	0	0	0.40%
18	1.500	500	0	0	0	0	0	0	0	0.00%
21	1.500	493	6	0	0	0	0	0	0	1.20%
30	1.500	497	3	0	0	0	0	0	0	0.60%
34	1.500	499	0	0	0	0	1	0	0	0.20%
38	1.500	498	2	0	0	0	0	0	0	0.40%
41	1.500	499	1	0	0	0	0	0	0	0.20%
50	1.500	499	1	0	0	0	0	0	0	0.20%
54	1.500	498	2	0	0	0	0	0	0	0.40%
58	1.500	500	0	0	0	0	0	0	0	0.00%
61	1.500	498	1	0	0	0	1	0	0	0.40%
70	1.500	499	0	0	0	0	0	0	0	0.00%
74	1.500	499	1	0	0	0	0	0	0	0.20%
78	1.500	496	3	1	0	0	0	0	0	0.80%
81	1.500	495	4	1	0	0	0	0	0	1.00%
90	1.500	497	3	0	0	0	0	0	0	0.60%
94	1.500	493	7	0	0	0	0	0	0	1.40%
98	1.500	474	26	0	0	0	0	0	0	5.20%
		496.60	3.10	0.10	0.00	0.00	0.10	0.00	0.00	0.66%

SPERM HEAD MORPHOLOGY

MALE	EMS (mg/kg)	NORMAL	NO HOOK	EXCESS. HOOK	AMORPHOUS	PIN-HEAD	TWO HEADS/TAILS	SHORT	OTHER	% ABNORMAL
4	100	498	1	0	0	0	0	0	1	0.40%
8	100	498	1	0	1	0	0	0	0	0.40%
12	100	499	0	0	0	0	1	0	0	0.20%
16	100	497	3	0	0	0	0	0	0	0.60%
24	100	500	0	0	0	0	0	0	0	0.00%
28	100	499	1	0	0	0	0	0	0	0.20%
32	100	499	1	0	0	0	0	0	0	0.20%
36	100	499	1	0	0	0	0	0	0	0.20%
44	100	498	2	0	0	0	0	0	0	0.40%
48	100	500	0	0	0	0	0	0	0	0.00%
52	100	498	2	0	0	0	0	0	0	0.40%
56	100	499	1	0	0	0	0	0	0	0.20%
64	100	480	20	0	0	0	0	0	0	4.00%
68	100	482	18	0	0	0	0	0	0	3.60%
72	100	500	0	0	0	0	0	0	0	0.00%
76	100	500	0	0	0	0	0	0	0	0.00%
84	100	490	10	0	0	0	0	0	0	2.00%
88	100	497	3	0	0	0	0	0	0	0.60%
92	100	499	1	0	0	0	0	0	0	0.20%
96	100	498	1	1	0	0	0	0	0	0.40%
		496.50	3.30	0.05	0.05	0.00	0.05	0.00	0.05	0.70%

Appendix III: Pathology Report, Organ Weights and Testicular Morphometry

Pathology Report

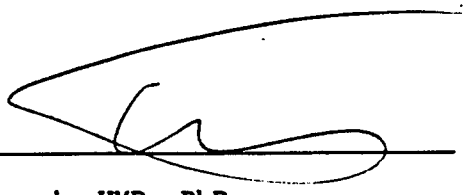
E-6579

INTRODUCTION:

One testis from each of the 20 animals in all five dose groups was embedded in glycol methacrylate and two replicate sections, each 2 microns thick, were cut. One, to measure planimetrically for tubule diameters, was stained by the periodic acid-Schiff technique. The other was stained with hematoxylin and eosin for histopathologic examination.

RESULT

There were no discernible abnormalities in any of the testes examined microscopically.



29 MAR 91

Thomas J. Bucci, VMD, PhD

Date

INDIVIDUAL ANIMAL TESTES:BRAIN WEIGHT RATIO

Dose Group 0.00 mg/kg

ANIMAL CID	BRAIN WGT	RIGHT TESTIS WGT	LEFT TESTIS WGT	TESTES:BRAIN WEIGHT RATIO
2011	2426	2016	2120	1.705
2015	2214	1926	1959	1.755
2019	2227	1869	1823	1.658
2023	2138	1987	1933	1.833
2031	2020	1825	1774	1.782
2035	2040	1727	1782	1.720
2039	2072	1551	1554	1.499
2043	2283	1824	1787	1.582
2051	2121	1667	1658	1.568
2055	2234	1812	1884	1.654
2059	2103	1709	1758	1.649
2062	2173	1593	1572	1.457
2070	2067	1688	1718	1.648
2074	2180	1721	1726	1.581
2078	2337	1863	1852	1.590
2082	2174	1712	1755	1.595
2090	1970	1698	1729	1.740
2094	2207	1737	1733	1.572
2098	2269	1851	2003	1.699
2101	2047	1557	1541	1.513
MEAN	**2165.10**	**1766.65**	**1783.05**	**1.640**
STDEV	**114.47**	**130.59**	**148.24**	**0.099**

INDIVIDUAL ANIMAL TESTES:BRAIN WEIGHT RATIO

Dose Group .375 mg/kg

ANIMAL CID	BRAIN WGT	RIGHT TESTIS WGT	LEFT TESTIS WGT	TESTES:BRAIN WEIGHT RATIO
2009	2113	1674	1704	1.599
2013	2132	1718	1689	1.598
2017	2099	1532	1590	1.487
2026	2165	1921	1984	1.804
2029	1845	1892	1926	2.069
2033	2189	1544	1676	1.471
2037	2101	1503	1509	1.434
2046	2222	1727	1775	1.576
2049	2214	1823	1870	1.668
2053	2287	1843	1908	1.640
2057	2157	1730	1765	1.620
2065	2075	1579	1715	1.587
2068	2228	1858	1953	1.711
2072	2140	1546	1570	1.456
2076	2272	1945	1974	1.725
2085	2419	1903	1853	1.553
2088	2110	1764	1718	1.650
2092	1997	1679	1685	1.685
2096	2209	1775	1772	1.606
2104	2096	1907	1912	1.822
MEAN	**2153.50**	**1743.15**	**1777.40**	**1.638**
STDEV	**116.93**	**144.30**	**140.21**	**0.146**

INDIVIDUAL ANIMAL TESTES:BRAIN WEIGHT RATIO

Dose Group .750 mg/kg

ANIMAL CID	BRAIN WGT	RIGHT TESTIS WGT	LEFT TESTIS WGT	TESTES:BRAIN WEIGHT RATIO
2008	1968	1564	1533	1.574
2012	2386	1518	1535	1.280
2021	2257	1962	1966	1.740
2025	2144	1804	1784	1.674
2028	2124	1672	1654	1.566
2032	2245	1844	1797	1.622
2041	2026	1716	1705	1.689
2045	2026	1754	1752	1.731
2048	2150	1665	1672	1.552
2052	2199	1877	1861	1.700
2064	2136	1639	1558	1.497
2067	2113	1813	1859	1.738
2071	2245	1811	1809	1.612
2080	2230	1849	1773	1.624
2084	2197	1882	1985	1.760
2087	2092	1675	1760	1.642
2091	2123	951	1847	1.318
2103	2033	1595	1620	1.581
MEAN	**2149.67**	**1699.50**	**1748.33**	**1.605**
STDEV	**102.94**	**222.96**	**134.45**	**0.134**

INDIVIDUAL ANIMAL TESTES:BRAIN WEIGHT RATIO

Dose Group 1.500 mg/kg

ANIMAL CID	BRAIN WGT	RIGHT TESTIS WGT	LEFT TESTIS WGT	TESTES:BRAIN WEIGHT RATIO
2007	2165	1771	1915	1.703
2016	2255	1689	1685	1.496
2020	1917	1921	1932	2.010
2024	2138	1603	1664	1.528
2027	2083	1584	1582	1.520
2036	2208	1614	1581	1.447
2040	2370	1935	1897	1.617
2044	2088	1879	1847	1.784
2047	2045	2014	1863	1.896
2056	2229	1975	2030	1.797
2060	2145	1861	1702	1.661
2063	2163	1791	1776	1.649
2066	2339	1875	1933	1.628
2075	2025	1568	1579	1.554
2079	2174	1796	2050	1.769
2083	2161	1679	1609	1.522
2086	2152	1489	1409	1.347
2095	2169	1687	1661	1.544
2099	2290	1836	1716	1.551
2102	2185	1765	1853	1.656
MEAN	**2165.05**	**1766.60**	**1764.20**	**1.634**
STDEV	**105.62**	**148.03**	**171.44**	**0.159**

INDIVIDUAL ANIMAL TESTES:BRAIN WEIGHT RATIO

Dose Group 100 mg/kg

ANIMAL CID	BRAIN WGT	RIGHT TESTIS WGT	LEFT TESTIS WGT	TESTES:BRAIN WEIGHT RATIO
2010	2028	1670	1639	1.632
2014	2235	1847	1951	1.699
2018	2158	1913	1871	1.753
2022	2157	1646	1735	1.567
2030	2112	1692	1738	1.624
2034	2138	1896	1871	1.762
2038	2059	1734	1734	1.684
2042	2168	2097	1945	1.864
2050	1933	1555	1577	1.620
2054	2278	1771	1808	1.571
2058	2379	1971	1960	1.652
2061	2272	1781	1840	1.594
2069	2192	1790	1848	1.660
2073	2164	1716	1819	1.634
2077	2524	1866	1947	1.511
2081	2061	1724	1743	1.682
2089	2212	1808	1867	1.661
2093	2263	1865	1993	1.705
2097	1904	1667	1689	1.763
2100	2144	1846	1858	1.728
MEAN	**2169.05**	**1792.75**	**1821.65**	**1.668**
STDEV	**141.96**	**125.62**	**113.51**	**0.082**

Individual Animal Average Diameter of Seminiferous Tubules

Treatment																				Group Mean
0.00 Lewisite	274.64	297.98	294.33	295.77	309.25	309.96	299.66	321.08	298.34	301.16	279.17	285.72	301.06	292.06	297.82	296.76	287.42	293.33	326.37	284.19	297.3±30.9
1.500 mg/kg L	298.20	297.04	311.91	310.11	285.41	295.82	289.17	309.06	289.00	293.47	293.36	269.22	288.20	281.80	314.21	270.53	300.65	289.15	295.77	295.55	293.8±27.7
100 mg/kg EMS	307.90	289.01	313.90	299.74	283.63	297.23	275.10	282.60	310.58	289.52	290.84	279.58	291.76	300.64	298.47	292.87	310.96	310.22	302.26	319.98	297.3±32.2

N=20 tubules/animal